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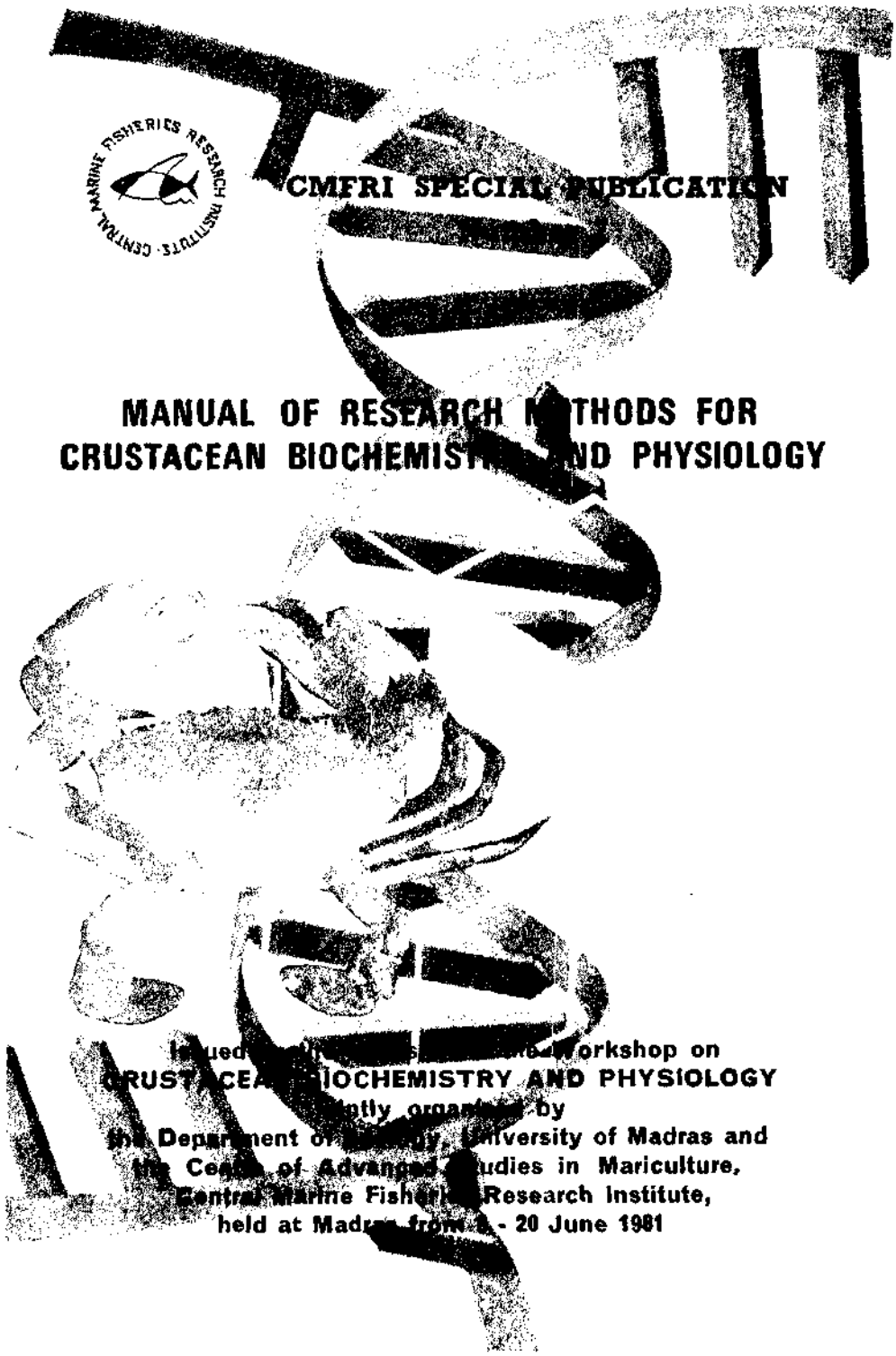
Number 7

MANUAL OF RESEARCH METHODS FOR CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY

Issued on the occasion of the Workshop on
CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY
jointly organised by
the Department of Zoology, University of Madras and
the Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
held at Madras from 8 - 20 June 1981



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Manual of Research Methods for Crustacean Biochemistry and Physiology

EDITED BY

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PHENOL OXIDASE * 9

9.1. Introduction

Phenol oxidase is an enzyme which is responsible for hydroxylation of phenols and dehydrogenation of O. diphenols into quinone. It is known to be distributed in the blood as well as in the cuticle of crustaceans (Summers, 1967). The enzyme can be assayed either spectrophotometrically, by measuring the dopa chrome formed (Preston & Taylor, 1970) or monometrically by measuring the oxygen consumed during the oxidation of the substrates (Hackman & Goldberg, 1967). The spectrophotometric method of assaying the enzyme of blood from *Scylla serrata* is presented here.

9.2. Phenol Oxidase Activity

9.2.1. Principle

Phenol oxidase oxidises the Dopa (diphenol) into brown coloured dopachromes. The formation of dopachromes can be determined by reading at 420 nm in a spectrophotometer (Preston & Taylor, 1970).

9.2.2. Reagents

1. 0.01 M Dopa in 0.05 M Tris—HCl buffer at pH 7.5.
2. Sodium dodecyl sulphate.

9.2.3. Procedure

9.2.3.1. *Enzyme preparations*

The haemolymph was collected in a cold tissue homogenizer. The clotted haemolymph was homogenized and centrifuged at 4000 rpm to remove the particulate material for 10 minutes using

* Prepared and verified by K. Nellaiappan, Department of Zoology, University of Madras, Madras-600 005.

a refrigerated centrifuge. The supernatant was used as enzyme source. To activate the enzyme, few crystals of sodium dodecyl sulphate was added in the enzyme to the concentration of mg/ml.

9.2.3.2. *Assay*

1. With 2 ml of substrate, add 0.2 ml of enzyme source. The increase in O.D. of the mixture should be noted immediately at 420 nm in a spectrophotometer.
2. Note the O.D. of the same upto 3 minutes for every 30 seconds interval.
3. Prepare the control with 0.2 ml of distilled water in 2 ml of substrate.
4. Determine the protein content of the sample by Biuret method (as mentioned in 6.2).

The results can be expressed as O.D./mg protein/minute.

9.3. Interpretation

It is known that the blood phenol oxidase of most of the arthropods is existing in the proenzyme state. The enzyme can be activated by artificial activators like detergents such as sodium dodecyl sulphate, sodium oleate, etc.

Though the phenol oxidase oxidizes a number of phenols, certain phenols are oxidized more effectively. Different substrates such as tyrosine, tyramine, phenol, cresol, dopa, dopamine protocatechuic acid, catechol, methyl catechol, Hydroquinone, pyrogallol, N-acetyl dopamine adrenaline, nor adrenaline, N-acetyl nor adrenaline can be used and based on the activity of the enzyme, a possible metabolic pathway can be suggested. In insect, it is reported that N-acetyl dopamine is the immediate precursor for tanning. However, in *Uca pugilator*, it is reported that N-acetyl nor adrenaline is the immediate precursor for tanning (Vacca & Fingerman, 1975). Substrate specificity and metabolic pathway of sclerotization of most of the other crustaceans as well as characterization of the enzymes asscoiated with sclerotization other than phenol oxidase in Crustacea have received little attention. Moreover it is known that in addition to tanning, the blood phenol oxidase is involved in defense mechanism (Brunet, 1980).

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